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The Genetics of Polycystic Kidney Disease
Part Three of Three

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Sequence repeats in the PKD-1 gene: implications for pathogenesis

I told you before in the beginning that there was a very funny aspect of the PKD-1 gene sequence. A little portion of it is illustrated here. If you go back a couple of slides it was as I showed you--As and Cs and Gs and Ts. This is just basically Cs and Ts. So it is really monotonous.

We think this may play a role in causing mutations. The reason is the following. PKD is the commonest major hereditary disease. It also has one of the highest de novo or spontaneous mutation rates. What I mean by that is where neither parent has the mutation and they have children who develop the disease de novo, so it's the first person in the family to get it. It is well, well recognized in this disease that it happens. It has been proven now molecularly that it happens. Old studies suggest that the rate that this happens in PKD is among the highest of any disease.

The third aspect to suggest that the PKD gene is very, very mutable, that is to say it is unstable, is the fact that virtually every single mutation that has been described, with a few exceptions, but virtually every mutation that has been described has been unique. If you compare that to, for instance, cystic fibrosis where over 70 to 80 percent of people have the same mutation and then they have a few others but most people have the same, in PKD almost every single mutation that has been discovered has been unique and independent, suggesting again it keeps going off, a lot, acquiring mutations a lot.

The fourth aspect of the disease that suggests that this particular gene is very, very unstable is the fact that we get so many cysts in the kidney. Most diseases that have this property of acquiring a mutation on top of an inherited mutation to cause disease--most diseases like that get a handful of tumors, for instance.

BRCA-1, the breast cancer gene--women get a handful of tumors; they don't get thousands to ten thousands of tumors. PKD is really quite unusual in the number of second hits or mutations that occur in a given individual. One to two percent, it is estimated, of all tubules, are affected, so one to two percent of a million tubules, acquire these mutations, so 10,000 times it goes off. This is hundreds to thousands of times higher than we see in most other diseases, again suggesting that this gene is really strange and has some unusual properties.

Sequence repeats in the PKD-1 gene may be responsible for its genetic instability: evidence from model systems

We think that this structure is one of those unusual properties that makes it go off. I won't go through the data, but there are other studies done by a number of other investigators that suggest structures like this but 100 times smaller than what we see in PKD-1 can cause mutations in model systems. So if something that is only 1/100th of the size that we
see in PKD can go off so much that they can actually see it in a model, we predict that this would do the same thing.

**How do sequence repeats such as those found in the PKD-1 gene cause genetic instability?**

As a matter of fact, in their model, the way this thing caused mutations would make a lot of sense for what we see clinically. In their model, the mutations would develop when the gene was transcribed, when an RNA copy was being made. The reason that is important is because most mutations are thought to occur typically when a cell is duplicating, when the DNA is being recopied. That's when the DNA is most vulnerable to getting changes introduced. In this model, cells don't have to be turned over. They can just be sitting there minding their own business, DNA can be just sitting there again minding its own business and develop a change. And how I mean that to be is illustrated in the next slide.

![Diagram of PKD gene and transcription process](image)

*Source: Germino GG, Hosp Pract (Off Ed) 1997 Mar 15;32(3):81-82*

**How CT repeats in the PKD-1 gene may cause genetic instability**

On the top part of this panel is just the PKD-1 gene. It is kind of a duplex, double-strand molecule here and it is just sort of a coil. This is what we think DNA looks like, this little coil. When DNA gets made into an RNA molecule which then makes protein, when it serves as its blueprint, there are special proteins, special molecules, that are necessary to make a copy. This is called RNA polymerase-2. The name is not important. The point is these little circles come, sit on DNA, and make a copy. They have to make a copy. But for it to make a copy, it has to unwind the coil. It has to get through it, so it has to unwind the coil of DNA. Again, there are special molecules that do that.
CT repeats cause a pause in unwinding of DNA during transcription
This CT repeat, Cs and Ts, is thought to create a funny structure which causes these proteins to stop. Normally they keep going along, they unwind, and they let the RNA molecules be made. This type of structure causes a pause. Again, there are repair systems that say, "Wait a minute. There's a problem here. We've got to fix it." It recruits these repair systems to the point where this pause is at, and it creates mistakes. So even though there really wasn't a mistake, after the repair systems get through, there is a mistake. It's like taking your car to the mechanic for one problem and ending up with a new problem. The same thing happens here we think.

The indistinguishable phenotypes of PKD1 and PKD2 suggest that their gene products may be partners of a common pathway

How the PKD-1 and PKD-2 genes may be functionally linked
Given that PKD-1 and PKD-2 look so much alike--they get the same cysts in the kidney,
same cysts in the liver, they get aneurysms, they all look the same—we wondered if perhaps the two genes, the proteins that they make, perhaps may function together in some way to make a link, like those hands holding each other together. So we've done some studies with Steve Somlo's group and indeed have shown that to be the case.

Proposed cartoon of PKD-1 and PKD-2 on a cell surface
This is our picture of what we think a kidney cell looks like with PKD-1 and PKD-2 proteins. Here is a better picture of the PKD-1 protein. It's a big, huge think we think. This is the part that sits inside the cell. This is the PKD-2 protein. We think these two proteins indeed really do form a connection. We think it's the two of these acting together that helps tell the cell how big it should be. If you break it, either by destroying this because of PKD-2 or destroying this because you have PKD-1, the information here does not get back to the brain part of the cell, the nucleus, and so the cell just keeps growing.

Is Mutation of PKD1 Necessary for All Forms of ADPKD?

- Incidence of three forms varies greatly
- The forms are essentially clinically indistinguishable

*We speculate that all forms of ADPKD may require inactivation of at least one copy of PKD1*
Possible role of acquired somatic PKD-1 mutations in causing PKD in patients with either PKD-1 or PKD-2 mutations

We wonder if perhaps PKD-1 is not playing a role in all the forms of PKD. PKD-2 is uncommon. It is 10 percent of the population that has PKD. PKD-3 is even more uncommon than that. It affects maybe 1 in 100 to 1 in 1,000 PKD families. It's really quite uncommon. There has only been maybe 10 families, if that, in the world that have been identified with this disease. And yet PKD-3 gets cysts in the kidney about as frequently as PKD-1. Since we believe you have to have a somatic mutation, an acquired mutation, to create the cysts, if the gene is really, really stable—that is to say it's not very common in the population--how would you get so many second hits?

One good model is if PKD-1 and PKD-2 or PKD-1 and PKD-3 join together and it takes just a reduction of either of either one or half of both to create a problem. Then you could imagine that you can have your first mutation, which you get from your parent, your PKD-1 or -2 or -3, and that's that; and then PKD-1, which is always going off because we know it is always going off acquiring mutations... even in normals it is going off, that perhaps in the right setting can break the link and cause a cyst to form. So we think that may be what goes on in ADPKD.

Why Make an Animal Model?

- Define pathogenesis of disease
- Evaluate role of gene product in normal development
- Study factors that affect expression of disease
- Test therapies
- Source material for establishing cell culture systems

New animal models of PKD

Reasons studies in mammals with short life spans such as the mouse can give us valuable information
For two minutes I just want to touch on one last part of the sort of genetic update. This is probably some of the most exciting stuff over the last year in the PKD field. That is the development of two animal models, two mouse models, of PKD disease. I know there is a lot of controversy about making animals and using animals to study human disease. I hope to make clear that there are some very specific purposes that cannot be done using cells or any other system that can only be done using animals.

Why do we want to make an animal, why do we want to use an animal model for a disease like this? Well, because they can help us study the disease through all stages. Humans live 60, 70, 80 years hopefully. We can't study that disease in the same person over 60, 70, or 80 years. Mice live two years. We can bring their life cycle into a very measurable time frame that we can actually analyze. Moreover, we can't do the sort of development processes, study how the PKD genes function during the formation of the kidney. There is good evidence to believe that the PKD gene is absolutely essential during the formation stages of the kidney. We cannot do those studies in humans. They can only be done using an animal, and mice are the most easy to use.

When we want to get into therapies, again looking at an individual, a human, we wouldn't want to give something that is potentially toxic with an unknown effect. We would like to know ahead of time that something is relatively safe and that it doesn't make the problem worse, instead it makes it better. Any therapy that we give to humans we may have to follow over 5 or 10 years to see a slight change. Whereas in an animal, again, you get a very immediate readout as to what we have done with our therapy. So there are many, many important reasons why an animal model is essential for studying particularly diseases like ADPKD.

**Appropriate animal models must be of a disease that acts like PKD in humans**

The problem is that in the past, although there have been many, many models of PKD and some fine work has been done using these models, none of them have disease that looks like the PKD that affects people. And so although there is some important information these models can provide, it is still not the same as studying the model that has what people get--PKD-1 or PKD-2.

Up to recently, there was nothing you could do about that. Now with genetic techniques and molecular engineering, you can actually reproduce in animals exactly what we see in humans. That is using the gene targeting techniques.
A new animal model using genetically altered mice

This is actually a picture I took from The New England Journal of Medicine, which tries to explain the process. I can only spend a second here explaining it. But basically we can harvest embryonic cells from mice and these can be grown in the laboratory. These are called ES cells, and that's shown on the top there. So these can be grown in the laboratory. These cells retain all the properties of a little, tiny embryo. So they can go ahead and grow up and become a kidney; they can become a gonad; they can become a brain; they can retain all the properties that you would want.

Another property of these cells though is that you can manipulate their DNA. As illustrated here, in your laboratory you can take a copy of your DNA, of the gene of interest, make a change, make a mutation of some sort for instance, take out an important part, and you can allow this piece of DNA to go into the mouse and replace the normal mouse gene. You can actually replace one copy with another. So you can manipulate it.

You then take those cells and you can inject them back into a little mouse embryo, it's called a blastocyst, put that back into a pregnant mouse, and then you get offspring, which are called chimeric. These mice are a mix of cells because you're injecting it into a fetus or embryo. There are already some cells there that are normal, but then there are the cells that you've added mutation. So you get an animal that is a mix of cell types.
Examples of the mouse model of PKD: chimeric mice
An example of that is illustrated here. This is our little chimeric mouse. If he wasn't chimeric, if he weren't a mix of cells, he would be all black. But because he has a mix of cells, he has some black, he's got a little bit of white, he's got a little bit of brown--he's kind of mixed all up.

These mice, then, can be bred. In their gonads, some of the cells will not have the mutation, and some will have the mutation. But when you breed them, if they happen to have had the mutation passed along to one of their offspring, that offspring then will be just like any person with an autosomal dominant condition. So half of that mouse's DNA will have one copy of their gene that now is mutated, just like it is in humans. This is what has been done for many, many genes. You read about it in the newspaper all the time, the different models that are made. And in the past year, this has been successfully done for PKD-1 and PKD-2.
The PKD-1 model in mice
PKD-1 was completed last year, last fall, by a group up in Boston. These mice they found indeed got cystic disease like in humans, with one exception. These animals when they had just one abnormal copy never got cystic disease. Only when you bred them so both copies were abnormal did you get cystic disease. So this model proves what we showed in humans. It takes two abnormal copies to get a cyst. Now the reason in humans you never see anybody with two abnormal copies is inherited is because we would all die. And that is what happened with these mice. The mice that had two abnormal copies all died because the kidneys become grossly full of cysts very, very early, while developing actually in utero and the animals died either during pregnancy or shortly thereafter. But it proves that PKD can cause cysts and loss of PKD, both copies, is required for a cyst to form.

The PKD-2 animal model is developed in mice
Steve Somlo's group just a couple of months ago now, in April, reported the same thing for PKD-2. They showed that if you inactivate, using the same technique, both copies of PKD-2, the mice die. However, they made another model which has a very interesting property. In this model, they had a mouse that has one mutated copy that is always mutated. So it is a fixed mutation. In the other copy, they made a dynamic mutation. What I mean by that is that it is unstable. Some times it's normal; sometimes it is not. So sort of like what we see in humans a little bit. These mice did not die. These mice survived. They were born. They have cystic disease. They look a lot like humans with cystic disease. They get cystic kidneys; they get cystic livers.

That is what is illustrated here. This is one of the cystic kidneys here of one of these mice that has this unstable gene combined with a non-unstable gene. You can see just a couple of cysts. Here is another animal, and you can see they have a lot of cysts. But those animals survived for a couple of weeks. Here is their liver. The liver also has a couple of
cysts. Finally, and perhaps most interesting is here if you look at this particular tubule, this is a tubule that came from an animal that has the unstable locus, so the unstable copy of the gene.

What you see very nicely here in real life is what I showed you in the cartoon in the beginning of the talk. Here is a normal tubule, here is the balloon coming out. The cells there acquired the mutation and ballooned out to create the cyst. So an acquired mutation can cause it. I won't go there because it's too long.

Concluding remarks
Let me just finish up with some summary comments since we are running out of time. Now we have two different animal models, the PKD-1 knock-out mouse proves the model that inactivation or loss of PKD causes the cyst phenotype and provides now a useful model for us to begin to think about how we might be able to replace PKD activity and protect cysts from forming.

The PKD model though has some limitations. It is hard to do therapy studies on dead animals. Unfortunately, virtually all of these animals died during the later stages of pregnancy. So we still have some way to go to make the model really like humans, where you have a copy that you inherit that is mutated and doesn't cause a problem, per se, but then you acquire mutations of the normal copy over time. Therefore, you get an animal that will perhaps live several weeks to months that you can then really properly test therapies. The PKD-2 model meets many of those criteria. So it is a very exciting model for testing some of the theories of the pathogenesis of the disease and potentially testing out some therapeutic applications.

I think, at that, I will close, since we are running over. Thank you for your attention.

References


